

# Purge-and-Trap Extraction of Geosmin and 2-Methylisoborneol from Soil

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## ABSTRACT

Although a number of techniques are available for estimating the biomass of filamentous microorganisms in soil, determination of their activity is much more difficult. We report on preliminary studies evaluating the potential use of the volatile microbial secondary metabolites geosmin and methylisoborneol as indicators of activity of filamentous microorganisms. The purge-and-trap technique for analysis of low concentrations of volatile organic compounds in air and water was tested for extracting the earthy-musty odor compounds geosmin and 2-methylisoborneol from soil. Two variations of this method were tried, one in which soil samples were placed in water for purging and another in which samples were purged with no added water. Volatile organic compounds purged from soil were collected on a porous polymer sorbent, Tenax TA. Naturally occurring geosmin and 2-methylisoborneol and added standards were successfully extracted from soil by both methods. Recovery efficiencies, however, were low. Results indicated that wet purging was the most efficacious extraction method yielding maximum recovery rates of added geosmin and 2-methylisoborneol standards of 15 and 24%, respectively.

GEOSMIN and 2-methylisoborneol (Fig. 1) are volatile organic compounds produced as metabolites of microorganisms and are important contributors to the earthy-musty odor of soil (Gerber and Lechevalier, 1965; Buttery and Garibaldi, 1976; Stotzky and Schenck, 1976). Both compounds are produced by certain actinomycetes (Gerber and Lechevalier, 1965; Gerber, 1979), cyanobacteria (Safferman et al., 1967; Tabachek and Yurkowski, 1976), and fungi (Mattheis and Roberts, 1992; Karahadian et al., 1985). Geosmin production has also been reported by amoeba (Hayes et al., 1991). However, the biomass of actinomycetes and fungi is far greater in most soils than that of cyanobacteria and amoebae, leading us to hypothesize that filamentous microorganisms produce the large majority of geosmin and 2-methylisoborneol in soil. Because these metabolites are readily degraded by some common soil bacteria (Narayan and Nunez, 1974) and, thus, probably do not accumulate in soil, they may be of value as indicators of activity of the organisms that produce them.

The purge-and-trap technique (Swinerton and Linnemom, 1967) is an efficient and commonly used method for the determination of low concentrations of volatile organic compounds in air and water. This type of system is commonly used for analysis of volatile organic compounds in the headspace of cultures of microorganisms. A modification of the purge-and-trap method, the closed-loop stripping system (Grob, 1973; Grob and Zurcher, 1976), has been used to determine amounts of geosmin and 2-methylisoborneol in water samples at levels below  $1.0 \text{ ng L}^{-1}$  (McGuire et al., 1981; Krasner et al., 1983).

The purpose of this study was to investigate the use of purge-and-trap methods for the extraction of geosmin

and 2-methylisoborneol from soil as part of an effort to determine their usefulness as indicators of activity of filamentous microorganisms. The specific objectives addressed in this study were: (i) extract added standards of geosmin and 2-methylisoborneol from soil to determine the efficiency of the purge-and-trap method, and (ii) attempt to extract naturally occurring geosmin and 2-methylisoborneol from soils.

## MATERIALS AND METHODS

Two soils were used in this study, a cropped soil and a relatively undisturbed woodland soil. The cropland soil is a Clarion loam (fine-loamy, mixed, mesic Typic Hapludoll) from the Iowa State University Ankeny Research Farm in Polk County. The woodland soil is a Lester loam (fine-loamy, mixed, mesic Mollic Hapludalf) collected from McFarland Park Nature Center in Story County.

Standards of geosmin and 2-methylisoborneol were obtained from Wako Chemicals (Richmond, VA). Both compounds were diluted in denatured ethyl alcohol before addition to soil or used as calibration standards. Purge-and-trap extractions were conducted on soils to which standards were added to make final concentrations ranging from  $10.0$  to  $0.01 \text{ mg kg}^{-1}$  soil.

Standards were added to soil samples by first placing the required amount of geosmin, 2-methylisoborneol, or both in  $5 \text{ mL}$  of denatured ethanol. The  $5\text{-mL}$  diluted aliquot was then slowly added to  $120 \text{ g}$  of soil in a  $500\text{-mL}$  beaker and mixed with a glass stirring rod. The beaker was then covered with aluminum foil and the sample was shaken vigorously for  $30 \text{ s}$  to thoroughly incorporate the added standards into the soil. Treated soil was then allowed to sit, covered, for  $45 \text{ min}$  before being divided into  $60\text{-g}$  portions for purging. Although the escape of some of the standards could be detected by smell at this time (the human nose is sensitive to these compounds in air at concentrations in the low parts-per-billion range), we believe the loss was insignificant.

Two methods for purge-and-trap extraction of geosmin and 2-methylisoborneol from soils were tested: (i) wet purging in which soil samples were placed in water for extraction, and (ii) dry purging where samples were placed in a chamber with no added water and purged. The apparatus used (Fig. 2) consisted of a sample chamber connected to a vacuum manifold. The sample chamber used for wet purging consisted of a tall form gas washing bottle (Krasner et al., 1983) and for dry purging a long glass tube ( $1 \text{ cm i.d.}$  by  $50 \text{ cm}$ ) was used (Fig. 2). Geosmin and 2-methylisoborneol purged from soil samples were collected in two in-line traps consisting of silanized glass tubes ( $11.5 \text{ cm}$  by  $4 \text{ mm i.d.}$ ) each loosely packed with Tenax TA (a porous polymer sorbent, Alltech Associates, Deerfield, IL) (Mattheis and Roberts, 1992), attached directly to the outlet of the sample chamber. The vacuum line was connected to the outlet end of the trap to create an air flow through the system, flushing volatiles from the sample and drawing them into the trap (tests of the efficiency of Tenax traps at capturing geosmin and 2-methylisoborneol are described below). Air entering the system was passed through an activated charcoal filter to prevent contamination of the sample.

For wet purging,  $500 \text{ mL}$  of  $\text{H}_2\text{O}$  and a magnetic stir bar were placed in a clean tall form gas washing bottle. Sixty grams of soil were then added to the water, the top of the

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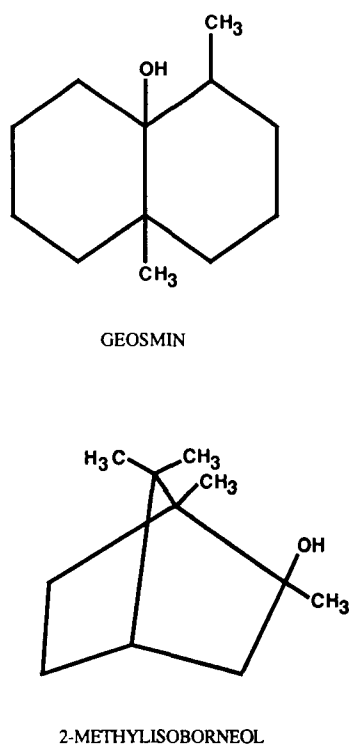


Fig. 1. Chemical structures of geosmin and 2-methylisoborneol.

bottle was replaced, and the bottle was placed on a stir plate. Vacuum pressure was applied to the system to create an air flow to purge volatile organic compounds from the soil and draw them into the Tenax trap.

Experiments were performed to compare the effectiveness of wet and dry purging systems at recovering added geosmin and 2-methylisoborneol from soil. The amount of geosmin, 2-methylisoborneol, or both recovered from identically treated soil samples by both methods was determined after purging for a period of 3 h (preliminary tests of purge times from 1 to 3 h indicated highest recovery after 3 h, data not shown).

After initial comparisons showed wet purging to be more effective than dry purging, we concentrated on improving recovery of geosmin and 2-methylisoborneol using the wet-purge system. Tests were conducted to determine the effect of length of purge period (3, 4, 5, 6, 7, 8, 9, and 24 h), temperature of purge water (23 and 50°C), and addition of NaSO<sub>4</sub> to the purge water (80 g L<sup>-1</sup>; Hwang et al., 1984) on the recovery of geosmin and 2-methylisoborneol.

Volatile organic compounds were desorbed from Tenax traps using a Supelco thermal tube desorber (Model 850, Supelco, Bellefonte, PA) and separated and quantified with a Hewlett-Packard gas chromatograph (Model 5890, Hewlett-Packard, Palo Alto, CA). Compounds were desorbed at 250°C for 5 min with a gas flow of 12 mL min<sup>-1</sup> and carried directly to the column of the gas chromatograph by a heated (170°C) transfer line. The gas chromatograph was equipped with a fused silica (DB-5) megabore column (30 m by 0.552 mm) and a flame ionization detector. The oven temperature program started at an initial temperature of 40°C for 10 min and then increased at a rate of 10°C min<sup>-1</sup> to a final temperature of 225°C for 15 min. The detector was operated at 280°C.

The identity of geosmin and 2-methylisoborneol isolated from soils to which no standards were added was confirmed by gas chromatography-mass spectroscopy using a Hewlett-Packard 5890 gas chromatograph coupled to a Hewlett-Packard

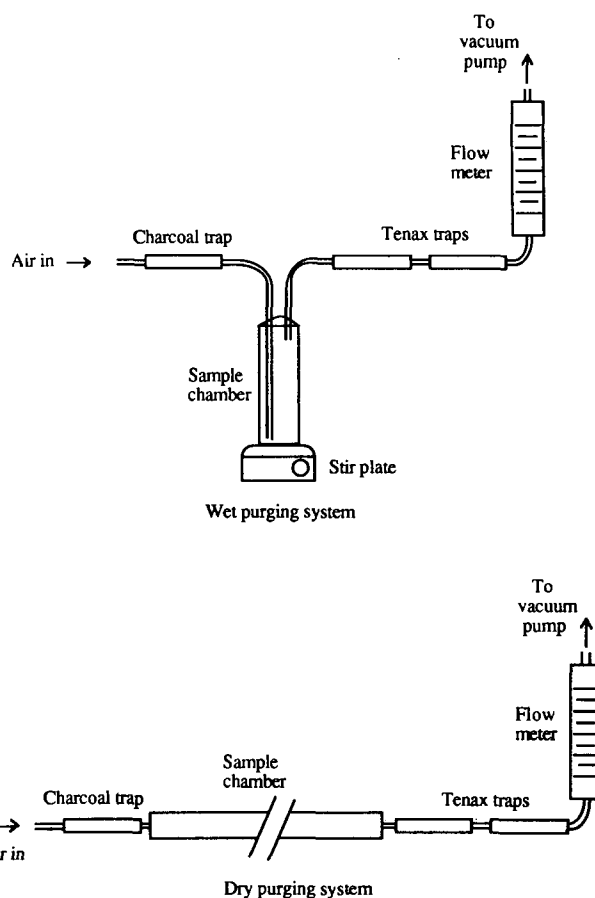


Fig. 2. Schematic diagram of the wet- and dry-purging systems.

5989 mass spectrometer. Gas chromatography methods were identical to those described above. The mass spectrometer was operated in the electron ionization mode with the source temperature at 250°C, ionization energy of 70 eV, and an electron multiplier voltage of 2200. The instrument was auto-tuned with perfluorotributylamine using mass 69, 219, and 502. The major molecular fragments for geosmin are mass 112, 97, and 125; the major fragments of 2-methylisoborneol are mass 95, 107, and 135.

Efficiency of traps at capturing and releasing geosmin and 2-methylisoborneol was tested by the following means. To determine the ability of Tenax to release captured geosmin and 2-methylisoborneol, known amounts of both compounds were injected directly onto Tenax in the trap with a long syringe. The treated traps were then subjected to the standard thermal desorption and gas chromatography analysis described above. As controls, the same known amounts were directly injected into the desorption chamber of the thermal desorption unit through a septum provided for this purpose. To examine the efficacy of the Tenax traps at capturing geosmin and 2-methylisoborneol, known amounts of both compounds were placed into empty sample chambers and subjected to purging for 3 h. Traps were then subjected to thermal desorption and gas chromatography analysis. Amounts of geosmin and 2-methylisoborneol recovered were compared with the controls. We tested the ability of one trap and two in-line and three in-line traps to capture all of the geosmin and 2-methylisoborneol placed in the sample chamber.

Lengths of actinomycete and fungal filaments in nonamended soil were estimated by direct microscopic counts using the

**Table 1.** Recovery efficiencies of geosmin and 2-methylisoborneol from treated 60-g soil samples using wet- and dry-purging systems.

| Amount added<br>mg kg <sup>-1</sup> soil | Amount recovered        |                        |                       |                        |
|--|-------------------------|------------------------|-----------------------|------------------------|
|  | Wet purge               |                        | Dry purge             |                        |
|  | Geosmin                 | 2-Methylisoborneol     | Geosmin               | 2-Methylisoborneol     |
|  | µg                      |                        |                       |                        |
| 0.5                                      | 4.11 ± 1.21†<br>(13.7%) | 5.19 ± 0.75<br>(17.3%) | 0.60 ± 0.30<br>(2.0%) | 6.30 ± 2.86<br>(21.0%) |
| 0.05                                     | 0.21 ± 0.03<br>(7.0%)   | 0.51 ± 0.13<br>(17.1%) | 0.06 ± 0.04<br>(2.0%) | 0.29 ± 0.15<br>(9.8%)  |

† Values given are mean ± standard deviation and, in parentheses, the recovery percentage.

membrane filter technique (Hanssen et al., 1974), calcoflour white for staining (West, 1988), and the grid line-intercept method for calculating filament lengths (Olson, 1950).

## RESULTS

Tests of the trapping and desorbing efficacy of our Tenax traps indicated that this system had an overall efficiency of 85% for geosmin and 74% for 2-methylisoborneol. Slightly >95% of both compounds was recovered when injected directly onto Tenax traps. Approximately 89% of geosmin standards placed in empty sample chambers was captured using two in-line traps, 96% of this amount on the first trap and 4% on the second. For 2-methylisoborneol, 78% of standards placed in the empty sample chamber was captured with two in-line traps, 60% of the total on the first trap and 40% on the second; only an additional 3% was recovered when a third trap was added.

Geosmin and 2-methylisoborneol were recovered from all treated soils by both of the purge-and-trap systems tested; however, only a portion of the amounts added were retrieved. Both compounds were consistently detected even at the lowest added concentrations (0.01 mg kg<sup>-1</sup> soil). Low levels of both compounds were occasionally detected in control soils to which only etha-

nol (5 mL) or nothing was added. There were no differences in recovery of naturally-occurring geosmin or 2-methylisoborneol from ethanol-added control soils and soils to which no ethanol was added.

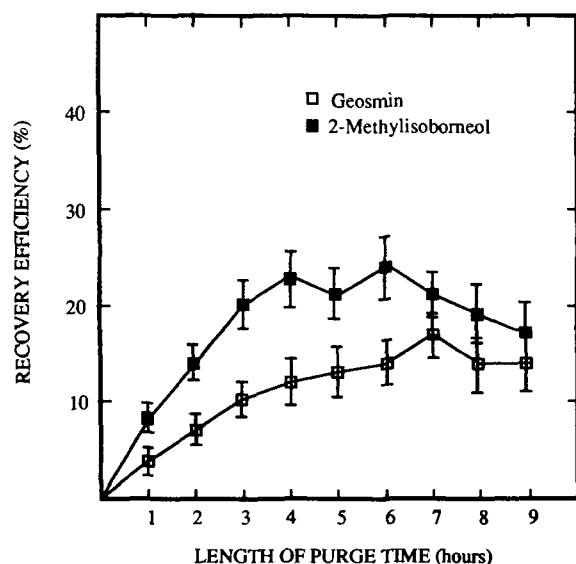
Comparison of the recovery rates of geosmin and methylisoborneol using the wet and dry purging systems is given in Table 1. At the high soil dosage (0.5 mg kg<sup>-1</sup> soil) wet purging appeared to be most effective for recovery of geosmin and dry purging for the extraction of 2-methylisoborneol. At the lower dosage (0.05 mg kg<sup>-1</sup> soil), however, wet purging was the most efficient method for recovering both compounds. Regardless of the purging method employed, 2-methylisoborneol was always recovered in greater quantities than geosmin.

Increasing the temperature of the wet-purging sample chamber from 23 to 50°C was found to have a negative effect on recovery of both compounds. For soils treated with both compounds at a concentration of 0.05 mg kg<sup>-1</sup> soil, recovery rates at 50°C were 4% for geosmin and 9% for 2-methylisoborneol, compared with 7 and 17%, respectively, at 23°C.

Addition of NaSO<sub>4</sub> to the purge water (80 g L<sup>-1</sup>) resulted in only a slight increase (2–3%) in the recovery rates of both compounds.

The length of time a soil sample was wet purged had important effects on the recovery of both geosmin and 2-methylisoborneol (Fig. 3). At an air flow rate of 30 mL min<sup>-1</sup>, the amount of geosmin extracted from a sample increased with up to 7 h of wet purging (maximum recovery, 15%). Recovery of 2-methylisoborneol increased with up to 4 to 6 h of purging, and then began to decline (maximum recovery, 24%).

Small amounts of geosmin and 2-methylisoborneol were extracted from both unamended soils using the wet-purge method (Table 2). More than 20 times as much geosmin and 2-methylisoborneol was extracted from the woodland soil than from the cropland soil. The amount of filamentous microorganisms was also much higher in the woodland soil (Table 2).



**Fig. 3.** Effect of purge time on recovery efficiencies of the wet-purging system. Data points are the means of three replications. Error bars = ± 1 standard error.

**Table 2.** Amounts of geosmin and 2-methylisoborneol extracted from untreated soils and direct microscopic counts of the length of fungal hyphae and actinomycete filaments.

| Soil     | Geosmin                  | 2-Methylisoborneol       | Fungal hyphae            | Actinomycete filaments   |
|----------|--------------------------|--------------------------|--------------------------|--------------------------|
|          | µg kg <sup>-1</sup> soil | µg kg <sup>-1</sup> soil | km kg <sup>-1</sup> soil | km kg <sup>-1</sup> soil |
| Woodland | 8.7 ± 0.75†              | 12.2 ± 1.95              | 385 ± 48                 | 149 ± 36                 |
| Cropland | 0.4 ± 0.36               | 0.6 ± 0.10               | 53 ± 47                  | 14 ± 21                  |

† Values given are mean ± standard deviation.

## DISCUSSION

Our results demonstrate that purge-and-trap methods can isolate both geosmin and 2-methylisoborneol from soil; however, recovery of these compounds is not highly efficient. The only other report of extraction of geosmin and 2-methylisoborneol from soil was that of Buttery and Garibaldi (1976) using a vacuum steam distillation technique. They recovered geosmin and 2-methylisoborneol on the order of 1.0 and 4.0  $\mu\text{g kg}^{-1}$  soil, respectively, but conducted no tests to determine the efficiency of their method. In comparison, 8.7  $\mu\text{g}$  geosmin and 12.2  $\mu\text{g}$  methylisoborneol  $\text{kg}^{-1}$  soil were recovered from the woodland soil using the purge-and-trap technique in our study. These are the highest values yet reported from soil.

When investigating the use of the purge-and-trap extraction for analysis of the geosmin and 2-methylisoborneol content of drinking water, Krasner et al. (1983) obtained mean recovery efficiencies of 112 and 97%, respectively, and detection limits of 2  $\text{ng L}^{-1}$  for both compounds. Low recovery of standards added to soil in our study is probably due to sorption of these compounds to soil surfaces. This is supported by tests of our trapping methods which demonstrate that, in soil-free systems, recoveries were relatively high. These findings also support our assumption that the loss of standards immediately after addition to soil was insignificant in that they do not escape from soil, especially dry soil, at a high rate. Geosmin was more readily adsorbed to Tenax traps than 2-methylisoborneol, as evidenced by the greater recovery from the empty sample chamber. However, the recovery rate of geosmin standards from soil was lower than that of 2-methylisoborneol, suggesting that geosmin may also be more strongly sorbed to soil. No reports were found in the literature concerning recovery efficiencies of geosmin and 2-methylisoborneol from substrates other than water for comparison. Water, however, may be the easiest substrate from which to extract these compounds because of their hydrophobic properties.

Recovery efficiency of 2-methylisoborneol using the wet-purging method was similar for soil at both treatment levels (0.5 and 0.05  $\text{mg kg}^{-1}$  soil, Table 1) suggesting that a linear relationship between concentration of 2-methylisoborneol in soil and recovery rate may exist, but this evidence is not conclusive. The recovery rate for geosmin was different at the two concentrations, indicating that recovery efficiency across a range of concentrations is not constant. Regardless, since geosmin and 2-methylisoborneol produced gradually in soil by microorganisms may behave differently than standards added at one point in time in a spike-recovery experiment, estimation of actual concentrations of these compounds in soil based on amounts recovered by the purge-and-trap method is probably not realistic.

Based on analysis of geosmin and 2-methylisoborneol standards applied directly to Tenax traps, the minimum detectable amounts of these compounds using the methods described above are 1.0 and 14.0  $\text{ng}$ , respectively. If the recovery efficiency of the purge-and-trap method

of extraction is 15% for geosmin and 24% for 2-methylisoborneol and 60-g soil samples are used for analysis, then, theoretically, the lowest detectable concentrations of these compounds in soil are  $\approx 0.2$  and 1.0  $\mu\text{g kg}^{-1}$  soil, respectively.

As a final note, it was encouraging to find that of the two soils analyzed for naturally occurring geosmin and methylisoborneol, the soil with the highest concentrations also had the greatest amounts of fungal hyphae and actinomycete filaments. Both of these compounds are metabolites, however, and their value may be as indicators of activity rather than biomass. Of course these are very preliminary results and further investigation will be required to determine the usefulness of geosmin and 2-methylisoborneol as indicators of the activity of filamentous microorganisms, especially considering the low extraction efficiency.

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